

Chiron Dkt. No.: 19720.004
(Atty Dkt. No. 072121-0274)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Erwin *et al.*

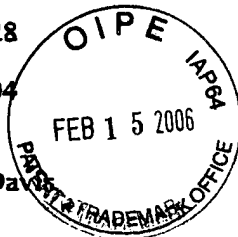
Title: ANTIBACTERIAL AGENTS

Appl. No.: 10/754,928

Filing Date: 01/08/2004

Examiner: Brian J. Davis

Art Unit: 1621



I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class mail in an envelope addressed to: Mail Stop _____, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 13 day of February, 2006.
By [Signature]

DECLARATION OF ERIC HARWOOD UNDER 37 C.F.R. § 1.132

Dear Examiner Davis:

I, DR. ERIC HARWOOD, state and declare that:

1. I am the inventor of the invention recited in at least claims 25-27. At the time of my invention, I was an employee of Pathogenesis, now Chiron.
2. Before the publication of the cited art, Kline *et al.*, *Journal of Medicinal Chemistry* (2002), 45(14), p. 3112-29, on June 7, 2002 (Web publication date), I conceived of the subject matter of at least claims 25-27 and 32 as evidenced by the attached Exhibits A and B.
3. Exhibit A includes copies of representative notebook pages documenting the synthesis of various compounds which are amino acid derivatives bearing a hydroxyalkyl side chain, a hydroxamic acid group, and a hydrophobic N-acyl group. Each of these compounds was synthesized for testing as an inhibitor of LpxC prior to June 7, 2002. Pages 35 and 42 show the initial synthesis of an exemplary compound of the claims. Pages 73, 77, 80, 83 and 90 show the synthesis of other representative compounds as part of library 1a. I conceived and synthesized each of the compounds in library 1a.
4. Exhibit B is an excerpt from an internal research report I prepared prior to June 7, 2002. The report documents the structures and synthesis of hydroxamic acids which I had prepared and proposed to prepare at the time as LpxC inhibitors. Among the compounds I prepared were compounds of library 1a which I had synthesized by the time of the report. The report also documents the compounds of library 2a which I had conceived of and knew how to make by the time of the report. Each "acyclic" compound (as distinguished

MADI_018340.1

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from cyclic oxazoline compounds) of libraries 1a and 2a is an amino acid derivative bearing a hydroxyalkyl side chain, a hydroxamic acid group, and a hydrophobic N-acyl group.

5. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the above-referenced application or any patent issuing thereon. All statements made of declarant's own knowledge are true and all statements made on information and belief are believed to be true.

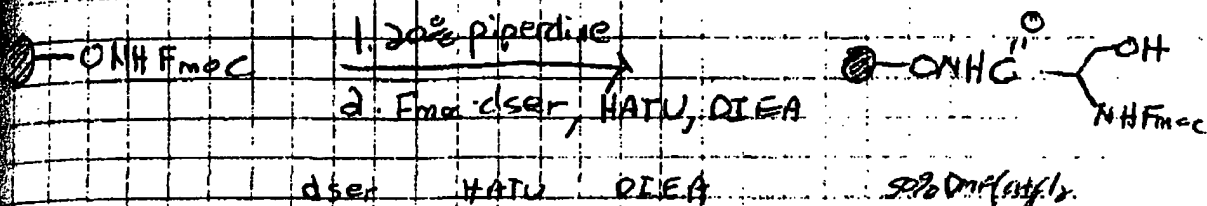

Eric Harwood, Ph.D.

13 Feb 06
Date

MADI_618346.1

Exhibit A

No. _____



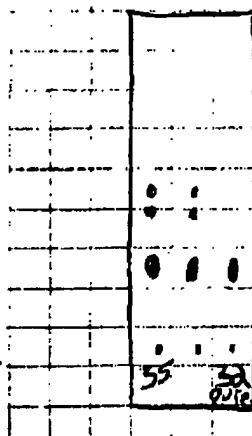
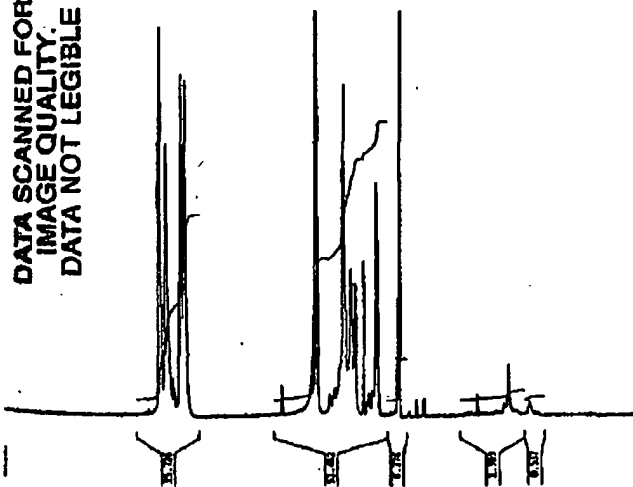
Reagent	Amount	Weight	Volume
Resin	1 mmol/g	327.3	380.2
DMF	500 mg	327.3 mg	380.2 mg
DMF	0.5 mmol	1 mmol	1 mmol
HATU	1 mmol	1 mmol	1 mmol
DIEA	1 mmol	1 mmol	1 mmol
DMF	10 ml	10 ml	10 ml

Resin was swelled for 5 hours in CH₂Cl₂. Treat with 10 ml 20% piperidine/DMF for 1 hour, Drain repeat 2 more times. Rinse 1x DMF, 3x CH₂OH, 3x CH₂Cl₂. In separate flask dissolve Fmoc-L-ser in 10 ml 90% DMF/CH₂Cl₂, add DIEA and cool to 0°. Add HATU in 1 portion, stir at 0°C for 1/2 hour. Add to Resin via syringe. Shake overnight.

7-19-00 Rinse 1x DMF, 3x CH₂OH, 3x CH₂Cl₂. HPLC → 768-35 → 569 mg

take 500 mg and cleave w/ TFA → 768-35-1 → 29 mg

DATA SCANNED FOR
IMAGE QUALITY.
DATA NOT LEGIBLE



25 mg after HPLC overnight
 ↓ Purify PTLC 70% MeOH/CH₂Cl₂
 12.7 mg pure
 Not soluble in acetone, good recryst?

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Date _____

Invented by _____

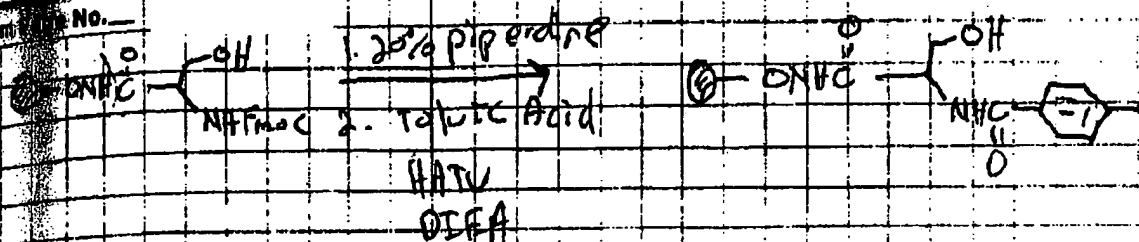
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Project No. _____
 Book No. 768 TITLE Coupling

Substance No. _____



	768-35	Toluic	HATU	DIEA
mol wt	136.15	380.2	139.25 (d=74)	
amount	400mg	82mg	228mg	122 ul
solvent	300mmol	600mmol	600mmol	700mmol

Swell CH_2Cl_2 & have. Treat 20% piperidine/DMF 3x.
 each. Rinse ~~DMF~~ DMF, CH_2OH , CH_2Cl , dissolve
 Toluic Acid in 8ml 50% DMF/ CH_2Cl and DIEA cool to 0°C.
 Add HATU stir 0°C 1/2 hour. Add to resin stir
 overnight. Rinse DMF, CH_2OH , CH_2Cl . ~~then~~

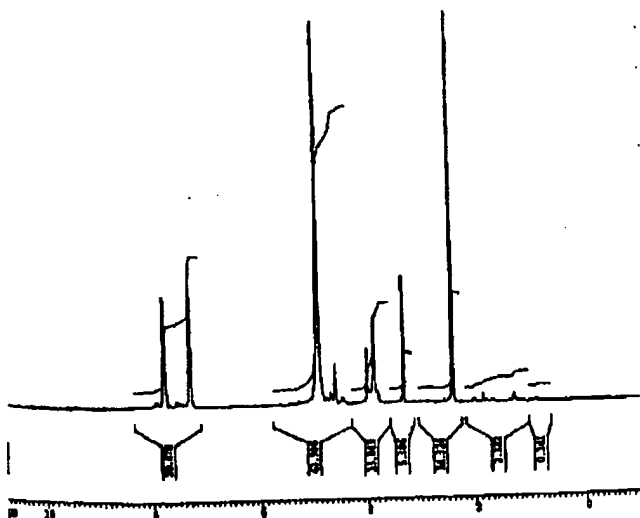
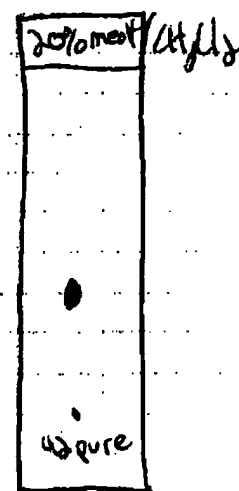
Leave 50mg INTFA. → 768-42

Purify PPLC 20% meth
 CH_2Cl obtain ~ 7.5mg

HPLC &
 MS are good

768-42-2

161 mmol/g



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Date

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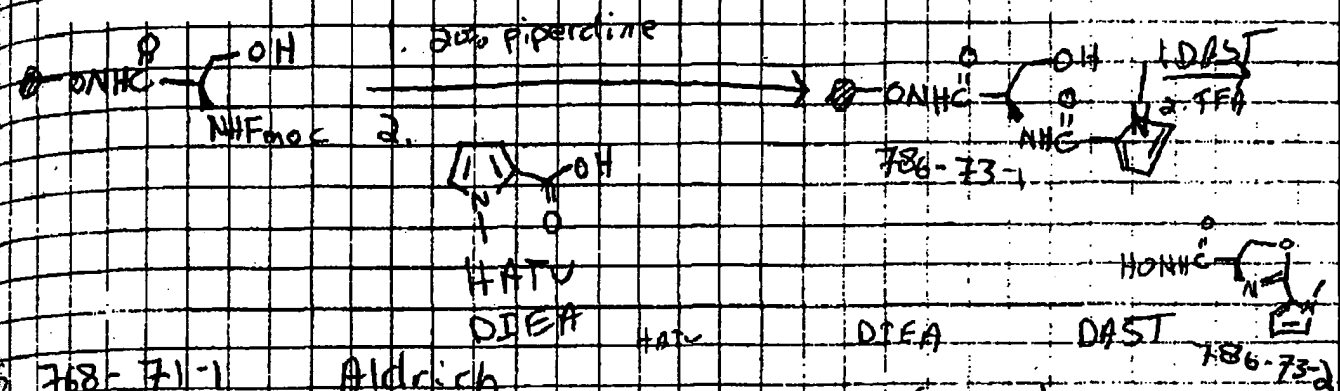
Date

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Page No. 71



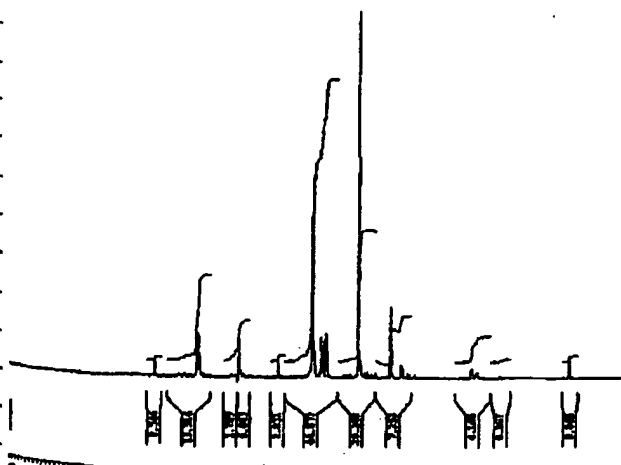
	768-71-1	Aldrich	HATU	DIEA	DAST	768-73-2
M.W. assume 1mmol/g	125.13	280.2	29.25(d=742)	161.19(d=228)		
Amount 450 mg	85 mg	257 mg	160 ul	150 ul		
Amount 1.5 mmol	1.675 mmol	1.675 mmol	90 mmol			

1) Swell in CH2Cl2 500 ml. Treat 3x w/ 20% piperidine/DMF 1 hour each; wash 3x DMF, 3x MeOH, 3x CH2Cl2. Remove 250 mg resin and swell in 500 ml CH2Cl2 (cool to 30°C (cooler setting) add DAST, stir 1 hour; wash 3x MeOH, 3x CH2Cl2.

768-73-1 → HPLC Shows some impurity at least 80% pure. M.S. messy, but shows m+m.

768-73-2 → NMR looks good minor impurities. HPLC not great. M.S. great. Submitted as is.

→ cleave 10% TFA. 768-73-1 = 4.6 mg. Remaining beads were 768-73-2 = 14.8 mg. also cleaved in 10% TFA.



To Page No. _____

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Date

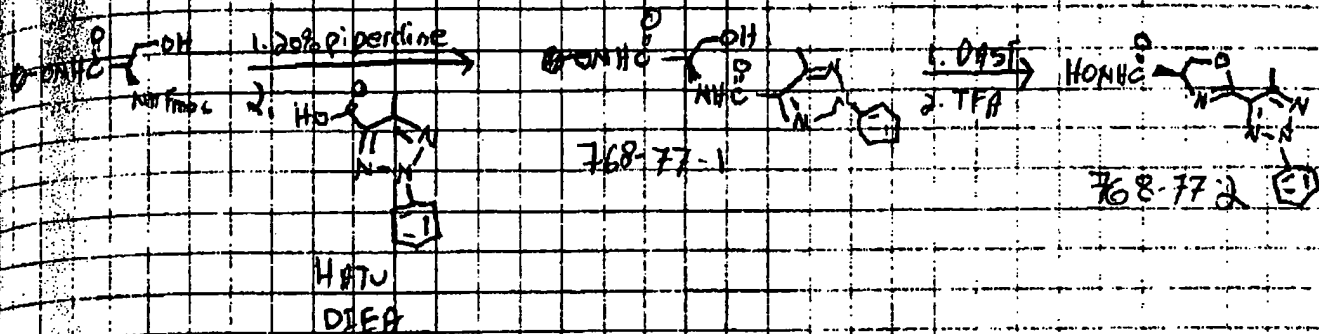
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[Signature: R. E. Harwood]

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Library la

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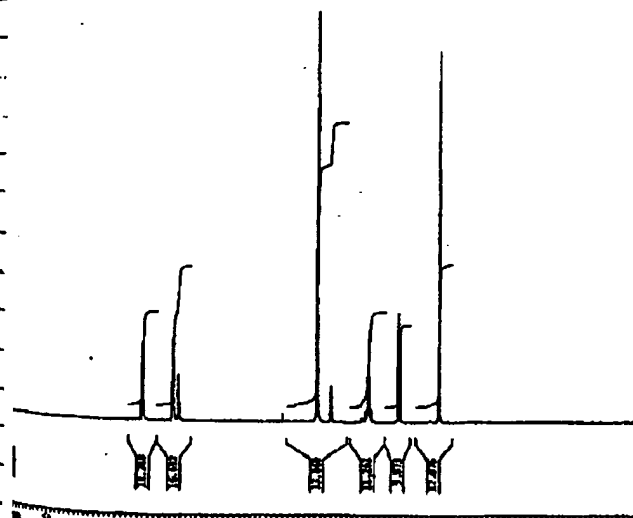
5 768-71-1

		HATU	DIEA	DAST
M.W. absorbance (mole/l)	203.20	380.2	109.25 (d = 700)	
amount 450 mg	137 mg	257	160 ul	
mmol .45 mmol	.675	.675	.90	

See 768-73 for prep

768-77-1 → HPLC great
M.S. good

768-77-2 → ¹H NMR OK
except extra signals
→ HPLC good
→ M.S. found
M+ 18



768-77-1 = 12.1 mg
768-77-2 = .6 mg

To Page No. _____

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Date

Invented by

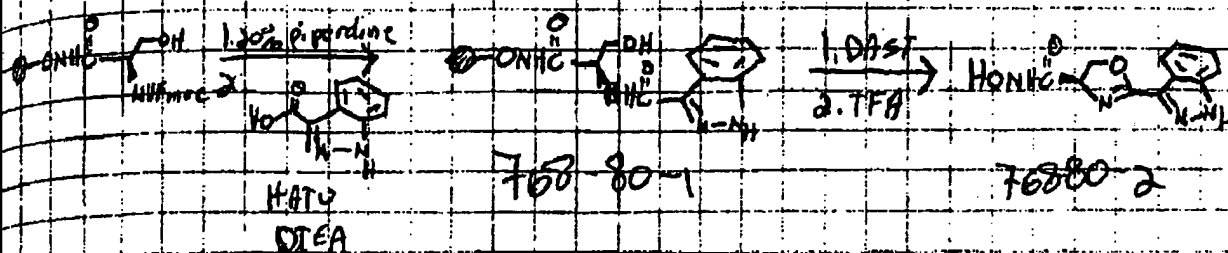
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Project No. _____

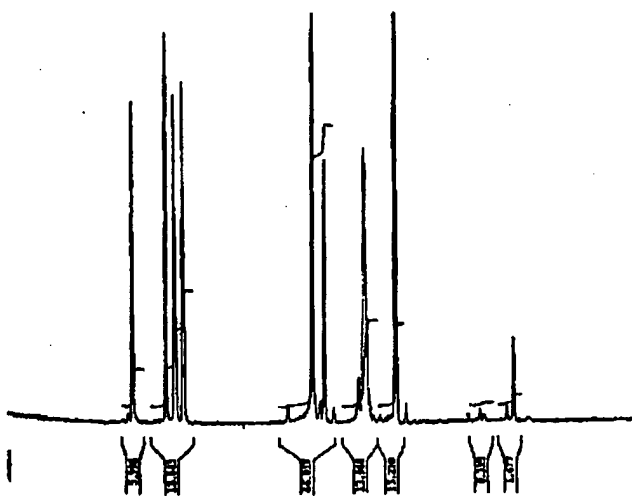
Book No. 768TITLE Library 1aFrom Page No. 7

S 768-71-1 Fluka DAST
 Mwt assume 1mmol/g 162.15 380.2 12.25 (74%)
 amount 450 mg 110 mg 257 mg 160 μ l
 mmol .45 mmol .675 .675 .90

ppt obtained when DIEA added

See 768-73 for prep

768-80-1 \rightarrow ¹H NMR good
 HPLC good
 N.S. good



768-80-1 = 9.2

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

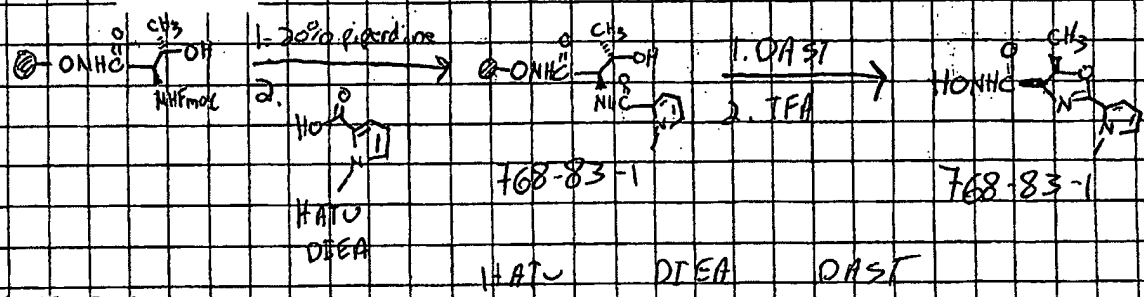
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From Page No. 72



S 768-72-1

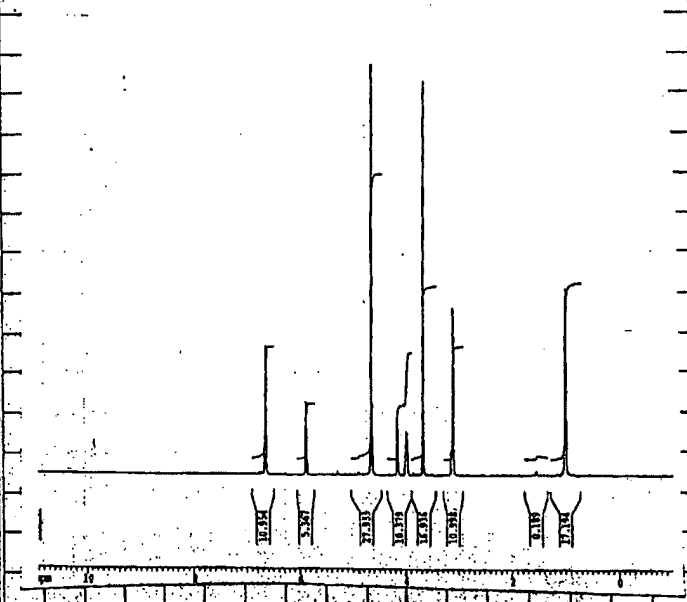
M.W. assume 1mmol/g	125.13	380.2	127.25 (742)
amount	150 mg	85 mg	257 mg
mmol	1.2 mmol	.675	.675

#11

see 768-73 for prep

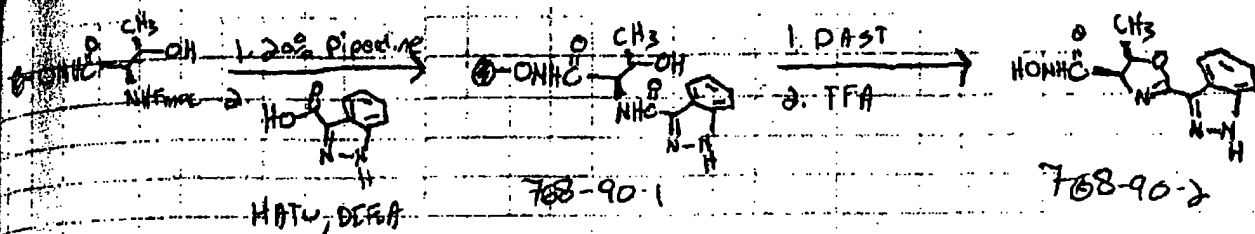
768-83-1 > 13 NMR good
 HPLC good
 Mrs. Found
 M + H +

768-83-1 = 66mg



Project No. 768 TITLE library 1a
 Book No. 768

Page No. 76

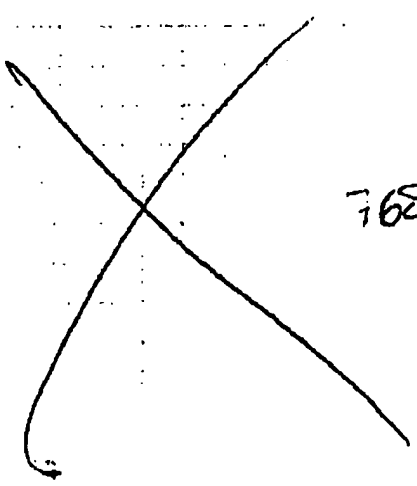
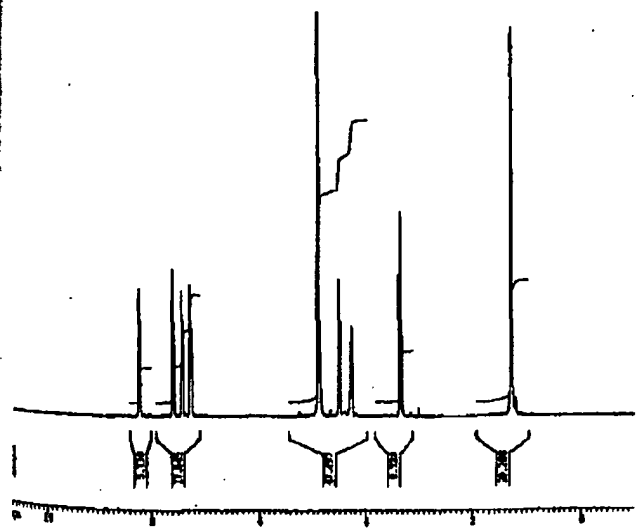


		HATU	DEEA	DAST
768-72-1	Fluka			
Wt assume 1mmol/g	16215	380.2	129.25 (742)	
Amount 430 mg	110 mg	257 mg	160 ml	
mol 4.5 mmol	1675	1675	90	

8 pfr when DAST is added

See 768-73 for prep

768-90-1 → 'HMR good
 HPLC good
 M.S. good



768-90-1 = 15.2%

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76800

Exhibit B

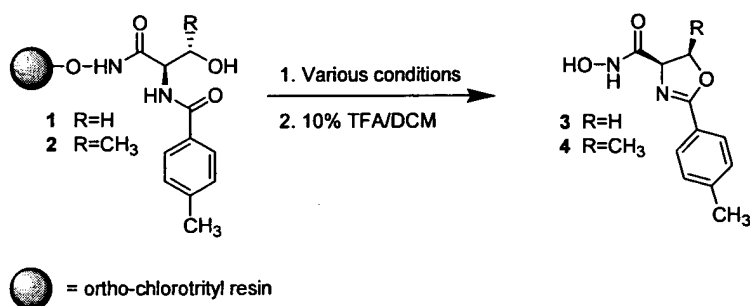
The First Library of LpxC-Directed Hydroxamic Acids

Synopsis

- ♦ Optimal conditions for the DAST cyclization were finalized.
- ♦ Library 1a was synthesized, purified, and characterized.
- ♦ Initial results using TBDMS-protected D-serine for library synthesis were obtained.
- ♦ Future Libraries were planned.

Main Points

The first research focus this month was to finalize the optimization of the cyclization of amido alcohols **1** and **2** to the desired oxazolines **3** and **4** (Scheme 1). Last month it was determined that the optimal conditions for conversion of **1** to **3** were 10 equivalents of DAST for 2 hours at temperatures between -20 and -30°C (results are summarized in Table I). However, these conditions resulted in an incomplete reaction and only an 18% yield for the conversion of **2** to **4**. Because of this result, the reaction was performed again on both **1** and **2** using 10 equivalents of DAST at -30°C but this time for a total of 8 hours to push the reaction to completion. It was determined by TLC that only a trace amount of either **3** or **4** was present at the end of 8 hours. Thus, it appeared that the excess DAST reagent was degrading the product over time. To test this hypothesis, a final reaction using **1** (**2** was no longer available in sufficient quantities) and 5 equivalents of DAST at -30°C for only 1 hour was performed. This resulted in a very clean, essentially quantitative conversion of **1** to **3**. We then used these conditions for the synthesis of library 1a.



Scheme 1. Cyclization of Amido Alcohols to Oxazolines

All the reactions were now optimized and the synthesis of the first library of LpxC-directed hydroxamic acids was carried out. The synthesis is outlined in Scheme 2 and has been discussed in previous monthly reports. As we analyzed the structure of the various intermediates on the synthetic pathway, we realized that amido alcohols **12** and **13** had very similar functionality to the desired oxazolines **14** and **15**. The amido alcohols are intermediates on the route to the oxazolines and all that is known about the LpxC binding site is that it requires a zinc binding motif (hydroxamic acid) and a hydrophobic group, thus we decided to screen both the acyclic and oxazoline hydroxamic acids as potential LpxC inhibitors. Therefore, using both D-serine and D-threonine and the 10 nitrogen heterocycles shown in Scheme 2 as the aromatic functionality results in a possible 40 compounds for screening.

Eric Harwood

Table I. Optimization of Oxazoline Formation

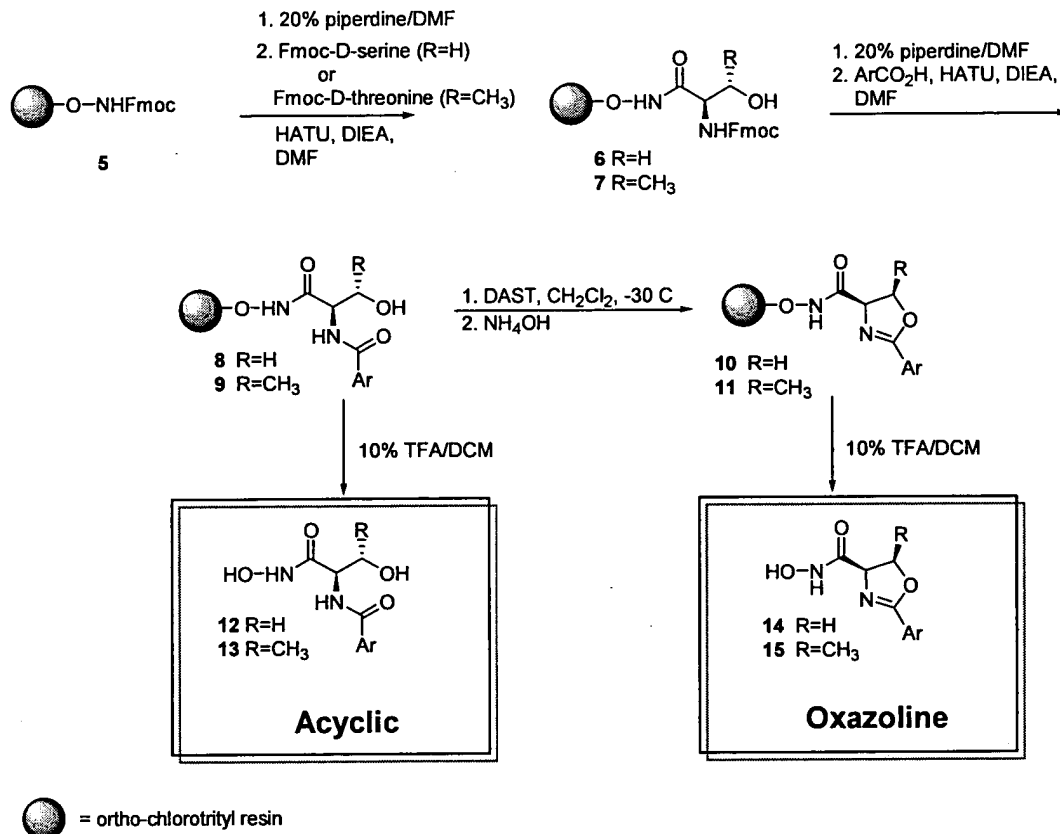
Amido Alcohol	Product	Conditions	Yield
1	3	DAST (10X), -30°C, 8h	Trace by TLC
2	4	DAST (10X), -30°C, 8h	Trace by TLC
1	3	DAST (5X), -30°C, 1h	100% !
1	3	DAST (10X), -30°C, 2h	35% (very clean)
2	4	DAST (10X), -30°C, 2h	18%* (very clean)
1	3	DAST (10X), -20°C, 2h	44% (very clean)
1	3	DAST (10X), -15°C, 2h	Trace by TLC
2	4	DAST (10X), -15°C, 2h	Trace by TLC
1	3	DAST (10X), 0°C, 2h	0%
2	4	DAST (10X), 0°C, 2h	0%
1	3	SOCl ₂ (10X), 0°C, 2h	0% (mess)
2	4	SOCl ₂ (10X), 0°C, 2h	0% (mess)

Library 1a was synthesized according to Scheme 2. The crude reaction mixtures were purified by means of pre-packed silica gel columns (1g) except in the case of difficult separations where either preparative scale TLC or HPLC was used as appropriate. Currently, 23 compounds have passed both an identity (MS and/or ¹H NMR) and a purity test (HPLC) and are ready for screening (Figure 1). Three more compounds await HPLC purification.

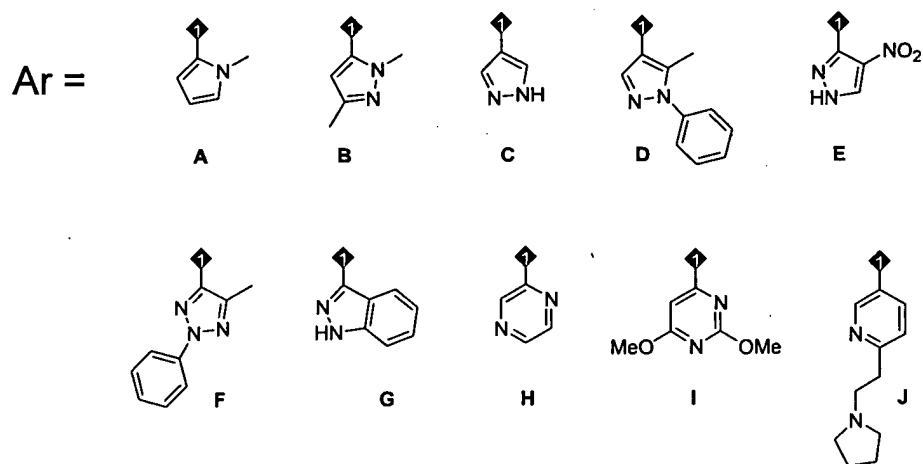
As can be seen in Figure 1, less than the theoretical number of compounds was obtained from library 1a. The synthetic conditions had been optimized using toluic acid as a model, however, the variety of reactivities among the nitrogen heterocycles caused some difficulties. Some of the heterocycles (C, E, and J) did not couple under the conditions determined for toluic acid resulting in no recovery of either the acyclic or oxazoline compound. Most of the other heterocycles that did couple also formed the oxygen/nitrogen bis-acylated product (O-acylation was discussed in the [REDACTED] report) as well as the desired N-acylated product (8 and 9). This not only decreased the yield of desired product, but also made purification quite difficult in some cases.

To avoid the problems associated with O-acylation with the next library, we decided to explore protecting the alcohol of serine as a *t*-butyldimethylsilyl (TBDMS) ether. Fmoc D-serine (16) was treated with TBDMS chloride and imidazole in DMF to obtain 17 as illustrated in Scheme 3. The protected D-serine derivative was then coupled to the solid support (5) using both HATU and PyBrOP as coupling agents to obtain 18. TLC analysis of the crude mixtures revealed only one major product (TFA treatment appears to have removed most of the silyl groups). Initial results of the coupling reaction with 17 appear to indicate that it is an improvement over the mixtures obtained when using 16. Using 17 will allow us to using a more powerful coupling agent (e.g. PyBrOP) and more equivalents of acid to get a higher coupling efficiency without worry of O-acylation. Before Library 1b is synthesized using 17, the synthesis will be completed with toluic acid to validate the coupling in the presence of the TBDMS group as well as removal of the TBDMS group with tetrabutylammonium fluoride (TBAF).

Eric Harwood



Library 1a (nitrogen heterocycles)



Scheme 2. Synthesis of Library 1

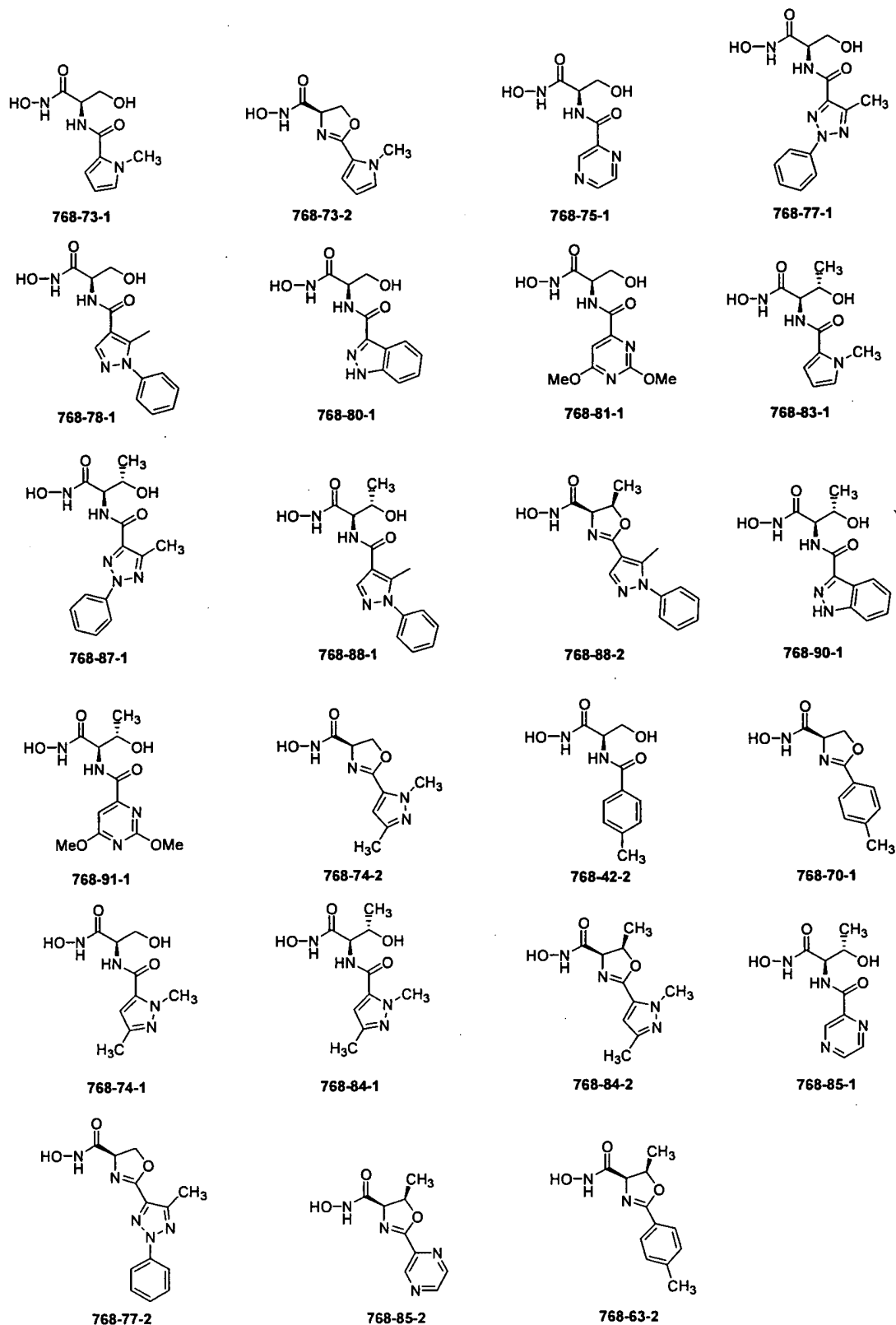
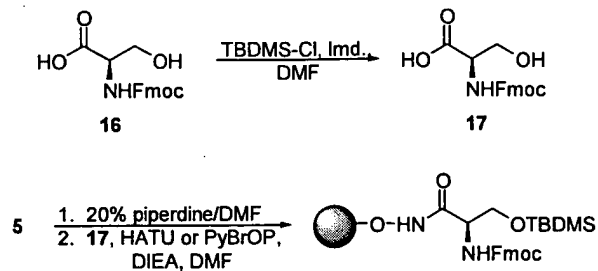


Figure 1. Compounds Ready for Screening

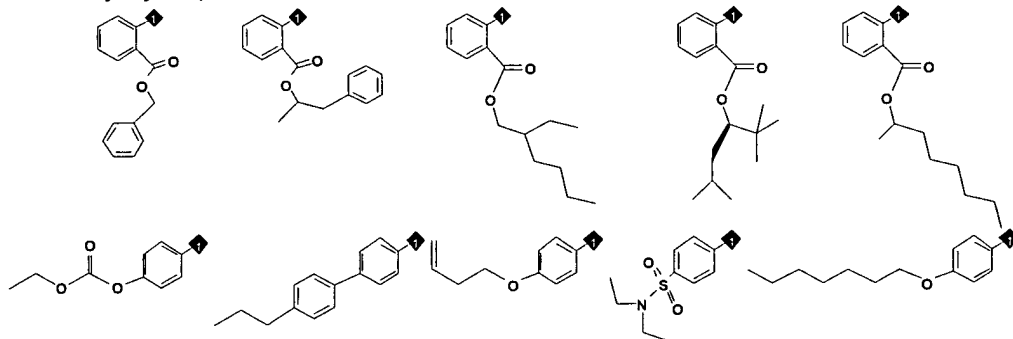
Eric Harwood



Scheme 3. Synthesis of and Coupling with a TBDMS Protected Serine

Library 1b (Figure 2) has been planned and will be carried out in the near future. For this second library we have chosen 20 aromatic acids representing two different types of compounds. The first ten acids have predominately hydrophobic contacts with long hydrocarbon chains to mimic that of the natural substrate for LpxC. The second ten acids represent a group with predominately polar and hydrogen bonding interactions. This library should give us some good SAR information and will be carried out in the next week after the synthesis with 17 is validated.

predominantly hydrophobic contacts



heterocycles: predominantly polar and hydrogen bonding interactions

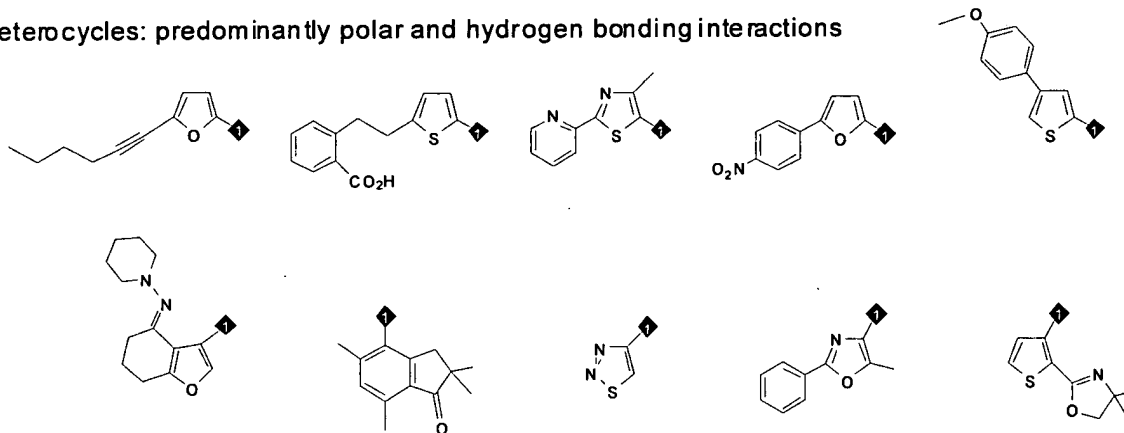


Figure 2. Proposed Aromatic Groups for Library 1b

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